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Otolith microstructure analysis for age determination of the Amazon characid *Triportheus albus**

by

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Abstract

Juvenile *Triportheus albus* (Characidae) were sampled with a ringnet in the Central Amazon floodplain between March and April 1993. The microstructure of the otoliths of *T. albus* was analyzed under the scanning electron microscope. The lapillus shows regular increments when ground in the sagittal plane and can be utilized for age determination. There are marks or checks formed at intervals of 14 rings, sometimes of 7 rings. Broad increments (>4.5 µm) rarely show subrings. The first 160 increments can be counted easily. In individuals which are bigger than 100 mm the microstructures at the edge are often undistinguishable. The calculation by counting of the increments yields an estimated daily growth of 0.426 mm ($p < 0.01$) for juveniles of *T. albus*.
Keywords: **Lapillus, otolith reading, age determination, *Triportheus albus*, Characidae, Amazonas.**

Resumo

Jovens *Triportheus albus* foram examinados após captura na Amazónia Central entre Março e Abril de 1993 com uma redinha. A microestrutura dos otólitos foi analisada com o microscópio eletrónico de varredura. O lapillus mostra as melhores microestruturas sendo lixado sagitalmente, e pode ser utilizado para a determinação de idade. Existem marcas ("checks") a intervalos de 14 e ocasionalmente de 7 anéis em quase todos os lapilli. Em incrementos muito largos (>4,5 µm) encontram-se raramente sub-anéis. É relativamente fácil proceder à contagem dos primeiros 160 incrementos. Em indivíduos maiores de 100 mm muitas vezes não é possível ver incrementos na borda. Da contagem dos incrementos pode calcular-se uma taxa de crescimento estimada de 0,426 mm por dia para juvenis de *T. albus*.

Introduction

The exact determination of age is fundamental for decisions in the fishery management. Fishes from temperate climate zones usually show good seasonal marks in the calcified

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parts of the body such as scales, otoliths, bones or spines. Due to the lack of great temperature changes in tropical regions, seasonal marks are difficult to detect and to validate.

PANNELLA (1971) worked on daily growth increments in fish otoliths and suggested to use them for a more precise age determination. Nowadays it is sure that the increments on the otoliths of teleostei are daily growth rings (CAMPANA 1992) and this method offers an important possibility for age determination in tropical fishes. Despite the high species number of fishes in Amazonia (GERY 1984) and their high commercial value (HONDA 1972), growth and population dynamics have been investigated for few fish species only (AMADIO & VILLACORTA-CORREA 1991; GODINHO 1994). The present study is a contribution to the age determination of one of the commercially important species, the tropical characid *Triportheus albus* (COPE). Fishes of the genus *Triportheus*, locally called "sardinha" (HONDA 1972), made up 704 t in 1976, which corresponds to 2,3 % of the total landing of the fishing vessels in Manaus (PETRERE 1978).

The aim of the present study was to determine which of the three otoliths, namely sagitta, asteriscus and lapillus, have useful microstructures for age determination. An estimation of the daily growth rate for juveniles of *Triportheus albus* is also worked out.

Material and methods

Individuals of the fish species *Triportheus albus* were sampled in the Central Amazon in the Rio Solimões floodplain, 39 km upstream from Manaus on the Machantaria Island ("Ilha de Machantaria"; 3°16'S, 60°01'W; Fig. 1).

Sampling took place between 9.00 and 18.00 hours on 26th March, 12th and 13th April 1993. A ringnet (15 m length, 2 m width, 50 mm meshsize) was used.

97 juveniles of *T. albus* (SL 53 mm to 119 mm; Fig. 2) were caught.

The heads were separated, put in small polythene bags and immediately frozen, as proven the best method for long term storage (GJOESAETER et al. 1984; BROTHERS 1987).

With a sagittal cut from the ventral side of the head, the otoliths were removed from the inner ear and cleaned mechanically with a brush in distilled water.

The maximal length and width of the lapillus and asteriscus were measured with a digital measuring computing system (model from CalComp, software: Sigma Scan 3.0).

The otolith preparation for examination of the microstructure with the Scanning Electron Microscope (SEM, Fa. CAMBRIDGE, stereoscan 90) followed the technical method as described by KARAKIRI & WESTERNHAGEN (1988). The otoliths were mounted on SEM stubs in the mounting medium Schellack and ground on a modified record-player. The grinding medium was laid on the turntable of the record player. The mounted otolith was fixed under the modified pick-up arm of the record player. To grind the otolith it was only necessary to let down the pick-up arm until the otolith reached the rotating grinding medium. The advantage of this method is the production of a planar ground surface. The grinding medium consisted of a silicium carbide water mixture (grain size 1, 2 or 3 µm). The grinding process was carried out until the nucleus was reached. For identification of the nucleus, otoliths were coloured with a solution mixture of gentian violet (crystal violet) and 5 % acetic acid (1:1) for 1 minute (KARAKIRI 1990; JANSEN 1991; CAMPANA 1992).

Subsequently, the otolith surface was treated with a 0.2 M EDTA (Ethylendiamin-tetraacetat) solution (pH 7.4) for 3 to 7 minutes. EDTA is a calcium chelator, which removes more calcium from the discontinuous zones than from the incremental zone, creating in the former a set of grooves and causing the latter to be slightly elevated (PANNELLA 1980; ZHANG 1992). This microstructure can be investigated under the SEM. After 24 hours drying at 60 °C, the otoliths were coated with gold (thickness: 25 nm) and examined under the SEM (15 kV).

It was impossible to measure microstructures along a defined radius because of irregularities of the otolith structure. Instead, the parts with the best structures were chosen and counts were made along adherent parallel radii.

Results

The shape of the otoliths of the examined characid is similar to other Ostariophysi (PLATT & POPPER 1981). The sagitta is long and delicate with thin longitudinal vanes (Fig. 3). It lies longitudinally in the lower part of the labyrinth organ (pars inferior). The sagitta is very fragile and cannot be used for examinations. The asteriscus is roughly circular and flat with dentated edges, which gives him the appearence of an "asterisk" (Fig. 4). The microstructures of the asteriscus consist of irregular and uncountable increments and is therefore useless for age determination.

The lapillus lies in the pars superior of the labyrinth organ (Fig. 5). It is dorsoventrad compressed with a slightly vaulted dorsal side and a convex ventral side. The longish sulcus on the ventral side covers three-fourth from the area of the otolith. The lapillus has the best microstructures of the three otoliths and can be used for age determination.

93 left and 93 right lapilli were measured. There were no growth differences between the left and the right lapilli (F-Test, $p > 5$ %). Therefore in the following descriptions the results are restricted to the left lapilli.

The best microstructure can be seen when grinding the lapillus in the sagittal-plane after grinding off the whole sulcus area. In relation to the position in the labyrinth organ the lapillus must be ground from the inside to the outside till reaching the primordium (nuclear area; Fig. 6).

The primordium is nearly spherical and situated acentrical, lateral in the front part of the lapillus with an average diameter of 24.4 µm (standard deviation: ± 2.1 µm). Under the SEM by 750-2000 x magnification regular and countable increments can be examined but only rostrad from the primordium (see Fig. 7). Caudad from the primordium the increments are broader with 2.5 µm to 11.7 µm (average: 7.3 µm) but irregular with disturbances and are not countable up to the edge (Fig. 7). Independent from which plane of observation, the microstructures become worse to the sulcus and only 45 % of the lapilli have good visible microstructures.

The relation between the growth of the radii from the lapillus and the standard length of *T. albus* is described by a linear regression ($p < 0.01$; Fig. 8). The lapillus grows caudad from the primordium (radius 2) better than rostrad (radius 1) in a relation 1:2.6.

Although the lapillus growths caudad better, the best microstructures are situated in the area of the 1st radius (Fig. 8). The first 15 to 40 (average 26) ring-structures are clearly defined with a width of the increments from 1,0 µm to 4.5 µm (average: 2.1 µm). The following increments vary also between 1.0 µm and 4,5 µm but they are with an average of 2.7 µm a little broader than the former ones. In lapilli with good microstructures it is possible to count the first 160 increments without problems. The following adjacent microstructures become more and more indistinct. Individuals which are bigger than 100 mm often have undistinguishable microstructures at the edge.

The interpretation of the microstructures is facilitated because of the existence of marks or checks formed every 14th ring and sometimes also at the 7th ring (Fig. 7). The checks are about 0.96 µm wide and are recognizable under the SEM as relatively

broad dark bands. Extremely broad increments could have subrings but they rarely appear so that they are not a problem for the interpretation of the microstructures.

Assuming that the increments are daily growth rings, the counting of the 18 individuals yields a daily growth of 0.426 mm ($p < 0.01$) for juveniles of *T. albus* with a standard length between 53 and 119 mm (Fig. 9).

Discussion

Each fish species has a specific shape of otoliths but the greatest differences exist between the ostariophysi and the other teleostei (SECOR et al. 1992). The examined characid *T. albus* confirms the similarity of the otoliths within the ostariophysi.

For *Colossoma macropomum* (Serrasalmidae) the lapillus has also good microstructures for age determination (VILLACORTA CORREA unpubl.). Otherwise WORTHMANN (1983) utilized the asteriscus and the lapillus for age analysis of juvenile "Pescadas" *Plagioscion squamosissimus* and *Plagioscion monti* (Scianidae). Therefore it is necessary to test every otolith before starting on age determinations.

Furthermore, different grinding planes must be examined, because otoliths grow irregularly (MORALES-NIN 1992). GAULDIE & NELSON (1990) observed that the sagitta of *Chrysophrys auratus* (Sparidae) grows slower to the sulcus and ventrad, where the otoliths lie on the sacculus. Against it, the otolith grows better and unrestricted to the dorsal edge. These observations can be confirmed for the lapillus of *T. albus*.

The fact that the lapillus grows caudad from the primordium faster than rostrad in relation 1:2.6 can be the reason for the worse microstructures in the caudal region. Caudad the lapillus needs more calcium and is therefore more affected by physiological disturbances. The laboratory experiments from MORALES-NIN (1987) with sea bass *Dicentrarchus labrax* and from PANNELLA (1980) with *Tilapia* indicate that high blood acidity levels of fish held under anoxic conditions cause reabsorbtion of calcium carbonate which lead to discontinuities in the process of mineralization. *T. albus* live in a biotope where most time of the year mid-day surface oxygen concentrations are only 0,5 mg/l. At night and in deeper water layers the oxygen concentrations are lower (JUNK et al. 1983). Oxygen deficiency could be an important stress factor which influences the process of mineralization of the otolith.

The average first 26 increments from *T. albus* are clearly distinguishable. For the "tambaqui" *Colossoma macropomum* are this the first 30 increments. The adjacent increments are more difficult to differentiate. PANNELLA (1980) explains these variations of the microstructures with changes of behaviour and alimentation during growth of the fish. But unlike the lapilli of *Colossoma macropomum* where the increments are only countable until the third month of life (equivalent to 90 days) (VILLACORTA CORRÊA unpubl.), the first 160 increments of the lapilli from *T. albus* are countable. WORTHMANN (1982) could count the daily rings also in adult "Pescadas" (*Plagioscion squamosissimus* and *Plagioscion monti*, Scianidae). So the pattern of growth increments seems to vary from one fish species to the other.

The lapillus of *T. albus* presents regular checks as relatively broad dark bands formed every 14th ring and sometimes also at the 7th ring (Fig. 7). Supposing the marks are caused by the moon phase, this could be an indication that the increments are formed daily. Checks have a higher amount of proteins than the discontinuous zone (ZHANG 1992) and are explained among other things as growth interruptions during new moon and full moon (CAMPANA & NEILSON 1985). WORTHMANN (1982)

established for the "Pescadas" growth interruptions every 14th day due to the lunar cycle. The Characoidei orient their spawning migrations in correlation with the moon phase (GOULDING 1979) and this is also observed for *Prochilodus platensis* (BAYLEY 1973). The lunar cycles seem to play a very important role for orientation of tropical fishes and are recorded in the otoliths.

Assuming the increments are formed daily the backdating of the oldest and youngest specimen of *T. albus* indicates that one must be born at the end of September and the other in January.

Accordingly, this species has a large spawning season in the Rio Solimões near Manaus. This presumption coincides with the observation of STOCKLER-PORTUGAL (1990) who caught a female full of eggs in October in the middle of the Rio Solimões near Fonte Boa and the yields of *Triportheus* are highest from August to October on the market in Manaus (PETRERE 1978). Furthermore GODINHO (1994) investigated the reproductive cycle of *Triportheus guentheri* in the Tres Marias reservoir (18°15'S, 45°15'W) São Francisco River, Brazil. He found out that *T. guentheri* has adhesive eggs and fractional spawning between November and February. Many *Triportheus* spp. seem to have a large spawning season.

In the present work a daily growth rate of 0.426 mm for juvenile *T. albus* was determined, which can only be considered as a rough standard value, because it cannot be proven that the growth increments are deposited daily, only few individuals could be investigated and larval and adult stages are missing. Juvenile *Plagioscion monti* exhibits a daily growth rate of 0.7 mm and *Plagioscion squamosissimus* of 0.5 mm (WORTHMANN 1982).

T. albus reaches sexual maturity at a standard length of 120 mm (SANTOS et al. 1984). Through counting the assumed daily increments from the longest exemplar of *T. albus* with 119 mm standard length it would take about seven months to become an adult fish.

Conclusions

It is assumed that the growth increments in the otoliths of juvenile *Triportheus albus* were deposited daily. Further investigation is necessary to prove this. To obtain a more exact growth curve it is necessary to include the larval and adult stages. This study analyses an estimated growth rate of 0.426 mm/day.

For tropical fishes it would be better to compare parallelly different calcified parts of the body such as scales, otoliths, bones or spines and to validate every method (BEAMISH & MCFARLANE 1987), because the increments of the otolith become worse as the fish gets older. *T. albus* specimen which were greater than 10 cm had already bad microstructures at the edge of the otolith. Otoliths are therefore useful for age determination of larval and juvenile fish. Complementary, scales could be used for age determination. WERDER & SOARES (1984) found out a 2-day rhythm of sclerite formation for *Brycon cf. melanopterus* (Characidae), *Semaprochilodus theraponura* (Curimatidae), *Semaprochilodus taeniurus* (Curimatidae), *Prochilodus nigricans* (Curimatidae), *Curimata cf. rutiloides* (Curimatidae) and a 1-day rhythm for *Colossoma macropomum (Serrasalmidae).*

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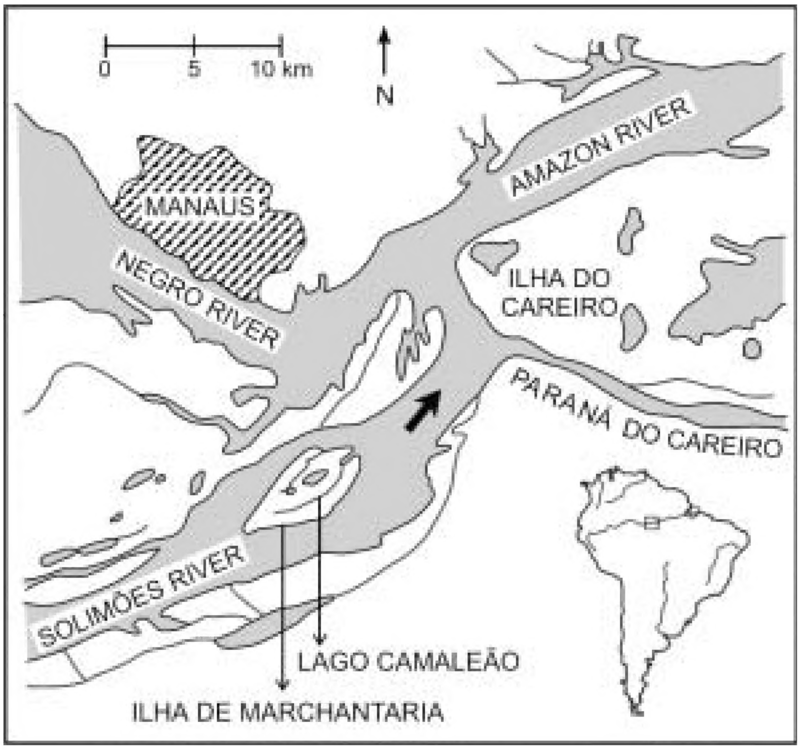

Fig. 1:
Ilha de Machantaria, Manaus, Brazil.

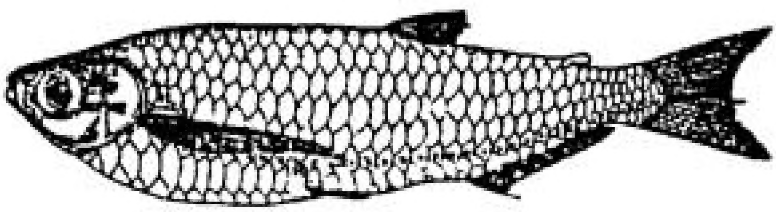

Fig. 2:
Triportheus albus COPE 1871 (1872).

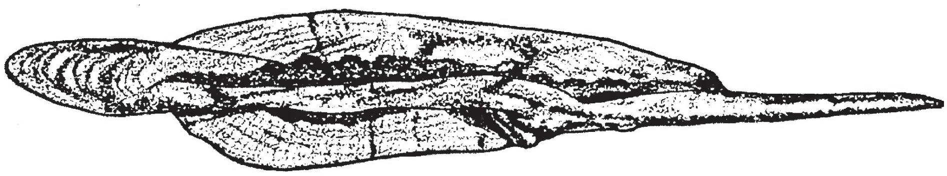

Fig. 3:
Otoliths of *Triportheus albus*. **A**: Sagitta (left), medial, (SL 6.8 cm), 38x. **B**: Sagitta (right), lateral, (SL 6.8 cm), 34x.

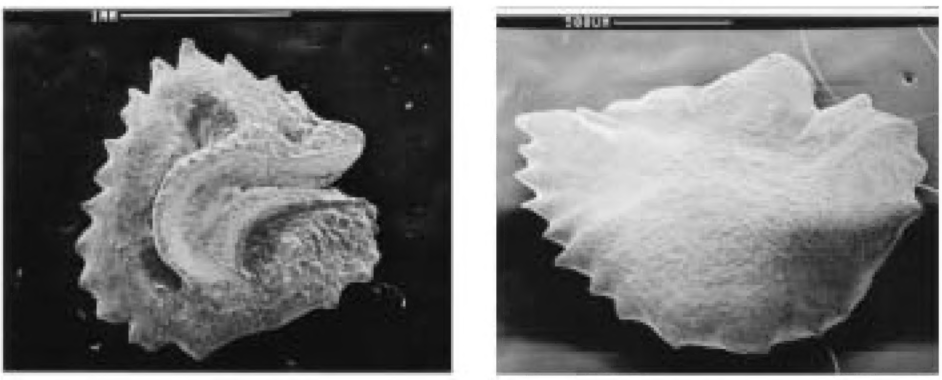

Fig. 4:
Otoliths of *Triportheus albus*. **A**: Asteriscus (left), lateral with sulcus, (SL 7.8 cm), 39x. **B**: Asteriscus (right), medial, (SL 7.8 cm), 54x.

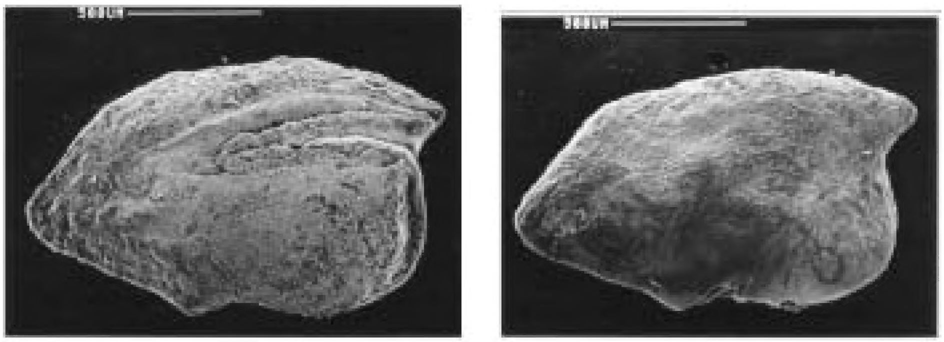

Fig. 5:
Otoliths of *Triportheus albus*. **A**: Lapillus (left), ventrad with sulcus (SL 7.8 cm), 61x. **B**: Lapillus (right), dorsad with sulcus (SL 7.8 cm), 63x.

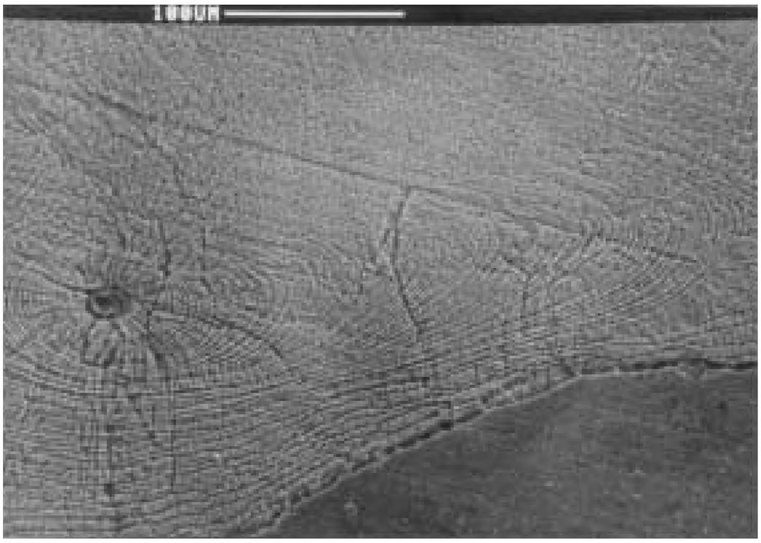

Fig. 6:
Lapillus with worst microstructures, area caudad from the primordium (dark hole), (SL 6.7 cm), 276x.

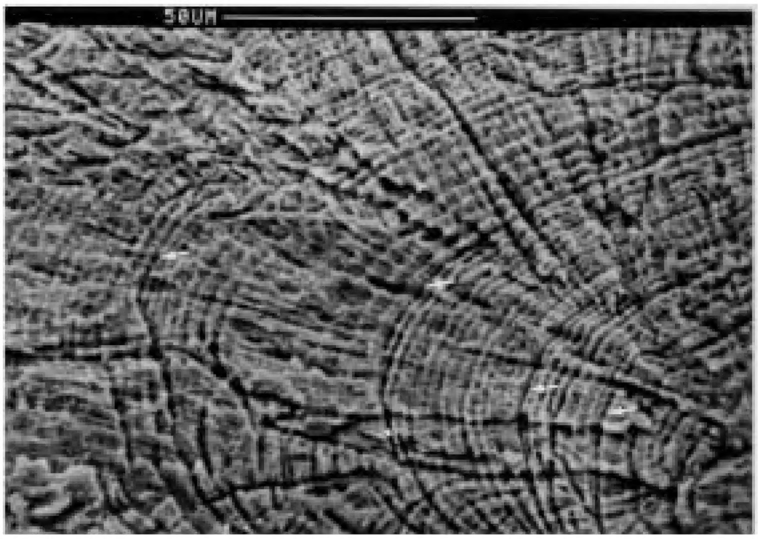

Fig. 7:
Lapillus, increments between checks formed at intervals of 14 rings, sometimes of 7 rings, 'checks' are indicated by the white arrows, (SL 7.1 cm), 786x.

A

B

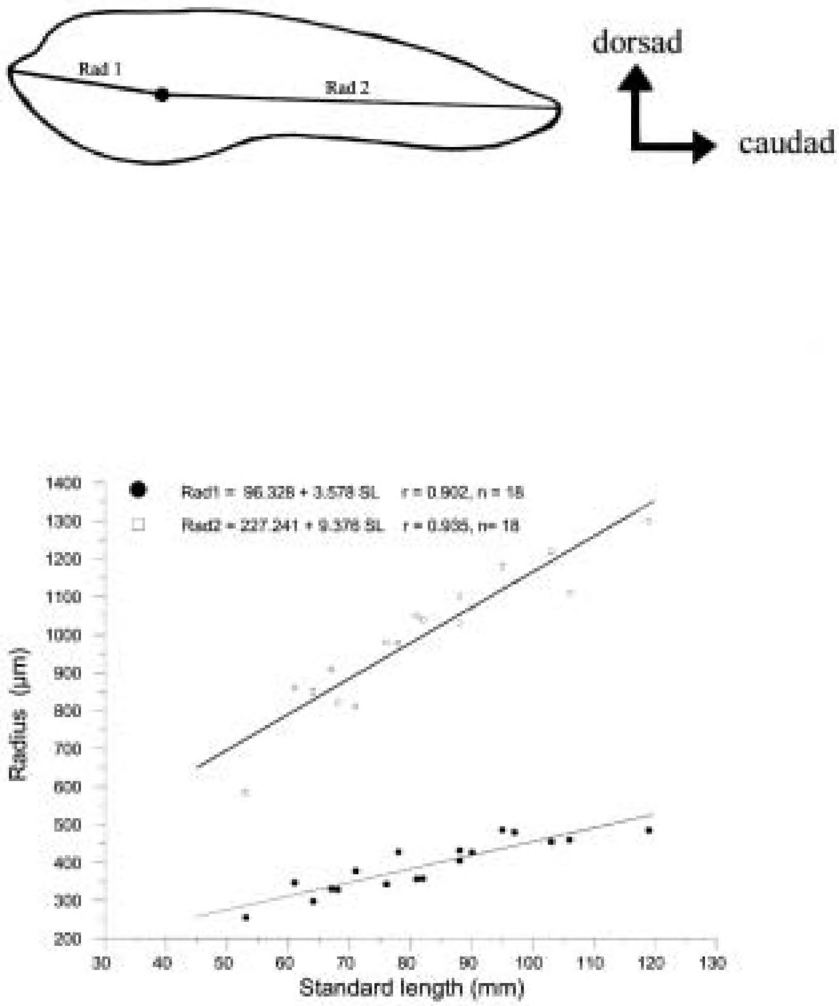

Fig. 8:
A: Position of radius 1 and radius 2 from the lapillus (sagittal, dark point = primordium). **B**: Relation between the growth of the radii 1 and 2 (Rad 1 and Rad 2) and the standard length (SL) of *T. albus*.

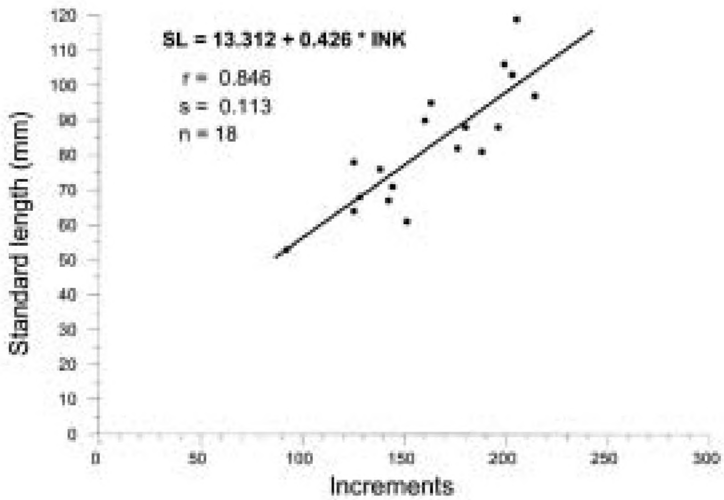

Fig. 9:
Relation between the number of increments (INK) and the standard length (SL) of *T. albus*.